AMERICAN JOURNAL OF PHYSICAL ANTHROPOLOGY 110:435–455 (1999)

Effect of Developmental and Ancestral High-Altitude Exposure on $\dot{V}O_2$peak of Andean and European/North American Natives

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KEY WORDS physical work capacity; human adaptation; Bolivia; exercise; migrant study design; physical activity level; hypoxia

ABSTRACT Peak oxygen consumption ($\dot{V}O_2$peak) was measured in 150 adult males (18–35 years old) in Bolivia, using a complete migrant study design to partition developmental from ancestral (genetic) effects of high-altitude (HA) exposure. High-altitude natives (HANs, Aymara/Quechua ancestry, n = 75) and low-altitude natives (LANs, European/North American ancestry, n = 75) were studied at high altitude (3,600–3,850 m) and near sea level (420 m). HAN and LAN migrant groups to a nonnative environment were classified as: multigeneration migrants, born and raised in a nonnative environment; child migrants who migrated to the nonnative environment during the period of growth and development (0–18 years old); and adult migrants who migrated after 18 years of age. Variability in $\dot{V}O_2$peak due to high-altitude adaptation was modeled by covariance analysis, adjusting for fat-free mass and physical activity (training) differences between groups. A trend for increased $\dot{V}O_2$peak with increasing developmental high-altitude exposure in migrant groups did not reach statistical significance, but low statistical power may have limited the ability to detect this effect. HANs and LANs born, raised, and tested at high altitude had similar $\dot{V}O_2$peak values, indicating no genetic effect, or an effect much smaller than that reported previously in the literature. There was no functional correlation between forced vital capacity and $\dot{V}O_2$peak, within or across groups. These results do not support the hypothesis that Andean HANs have been selected to express a greater physical work capacity in hypoxia. Am J Phys Anthropol 110:435–455, 1999.

The extent to which structural or functional components of the oxygen transport system show genetic adaptation in the Andean high-altitude native (HAN) has not been determined. While many unique aspects of the HAN phenotype have been identified in both Andean and Himalayan populations (e.g., Beall et al., 1994; Greksa, 1994; Kayser et al., 1991; Vincent et al., 1978; Hochachka, 1991; Reynafarje and Velasquez, 1966; Mazess, 1969; Lahiri et al., 1967; Lahiri and Milledge, 1966; Frisancho et al., 1973a,b, 1995, 1997; Hurtado, 1964; Sun et al., 1990: Droma et al., 1991; Zhuang et al., 1993; Schoene et al., 1990; Boyce et al., 1974), most previous study designs have been insufficient to partition the effect of ancestral high-altitude exposure (genetic ad-

Grant sponsor: NSF; Grant number: SBR-9523454; Grant sponsor: Latin American Studies Program, Cornell University; Grant sponsor: Cornell Institute for International Food, Agriculture, and Development.

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Received 31 August 1998; accepted 1 August 1999.

aptation) from the effect of high-altitude exposure during the period of growth and development (developmental adaptation). At the phenotypic level it is possible to make inference to genetic adaptation if a migrant study design is employed (Harrison, 1966). The migrant design allows adaptive components (developmental vs. genetic) to be partitioned, based on a comparison of study groups defined by ancestral exposure to high altitude and migration status to a new or nonnative environment. Versions of the migrant study design have been applied to study the peak oxygen consumption ($\dot{V}O_2$peak) during maximal exercise and the lung volume of Andean HANs because these two measures are presumed to have functional importance related to oxygen transport. Lung volume is seen as a proxy measure of the pulmonary diffusion capacity (Cerney et al., 1973; DeGraff et al., 1970; Dempsey et al., 1971; Guleria et al., 1971; Johnson et al., 1985), while $\dot{V}O_2$peak at high altitude is seen as a measure of the integrated functioning of the entire O_2 transport system (Buskirk, 1976; Frisancho et al., 1995). In particular, the $\dot{V}O_2$peak has received attention because the physical work capacity lies within a functional realm that may be proximally related to measures of Darwinian fitness such as fertility and mortality, and as such the $\dot{V}O_2$peak phenotype may have come under past selection pressure in the Andes (see Mazess, 1973).

Previous migrant studies indicate both developmental and genetic components to explain the larger lung volume (Frisancho et al., 1973a, 1997; Greksa et al., 1988; Greksa, 1988; Stinson, 1985) and higher $\dot{V}O_2$peak (Frisancho et al., 1973b, 1995) of the Andean HAN. However, some of these studies can be criticized on the basis of 1) problems with population admixture, and/or 2) problems with confounding, i.e., differences between comparison groups in other factors known to influence the phenotype difference being measured. Admixture problems can be minimized by the careful designation of study subjects into specifically defined study groups, but the problem of confounding is more intractable. In particular, with respect to the $\dot{V}O_2$peak measurement, previous studies have not properly accounted for variability between study populations in physical activity level (training), and/or have failed to appreciate the importance of properly scaling $\dot{V}O_2$peak to body size.

Here, $\dot{V}O_2$peak differences between eight male subject groups are reported (including HANs and European/North American natives defined according to the migrant study approach). This paper complements a previous paper (Brutsaert et al., 1999) where the pulmonary function characteristics of these same subject groups were described, and where it was concluded that both developmental and genetic effects explain the larger forced vital capacity (FVC) found in Andean natives.

Because of the potential for confounding when describing $\dot{V}O_2$peak differences between groups due to high-altitude adaptation, a covariance model approach is used to control for known population differences in body size, body composition, and training level. The covariance approach is considered essential, given two specific problems seen in previous studies. Firstly, most previous studies describing the HAN vs. low-altitude native (LAN) $\dot{V}O_2$peak difference have used the ratio standard approach to control for body size and body composition differences between groups. In the ratio standard approach, the $\dot{V}O_2$peak is divided by some component of the body size to give $ml \cdot min^{-1} \cdot kg^{-1}$ body weight or $ml \cdot min^{-1} \cdot kg^{-1}$ fat free mass. This approach introduces bias by favoring individuals of small body size (Nevill et al., 1992; Tanner, 1949).

Secondly, previous studies have not sufficiently controlled for physical activity level differences between groups. In Andean studies in particular, HAN groups have generally been rural subsistence farmers with high levels of physical activity, while European/North American comparison groups have generally been urban dwellers with variable activity patterns. From extensive submaximal exercise testing conducted with each subject, we have constructed an index of training level (physical activity). The index is based on the well-known principle that the heart rate (HR) recovery after exercise is more rapid and more complete in a well-trained individual compared to an nontrained individual (Cardus and Spencer,

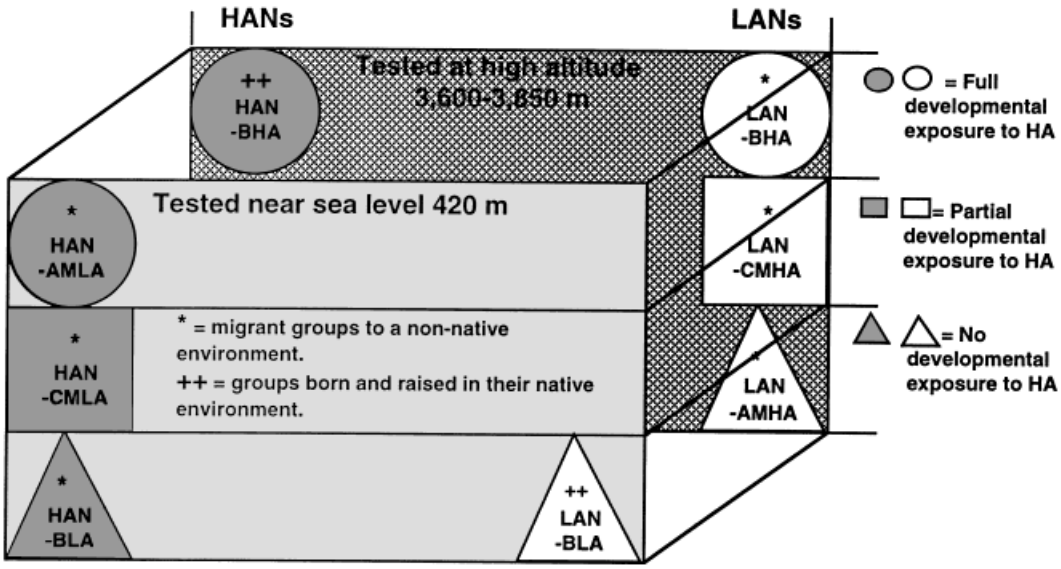

Fig. 1. Study design. Eight groups of subjects were studied. Subjects were defined based on 1) ancestry (high-altitude native, HAN; low-altitude native, LAN), 2) migration status (which defines the level of developmental exposure to high altitude) full, partial, or no exposure during growth and development, and 3) the environment in which they were tested (420 m = Santa Cruz, Bolivia; 3,600 m = La Paz, Bolivia).

1976; Kirby and Hartung, 1980; Darr et al., 1988). The index is used as a covariate to remove the effect of training in those statistical models that endeavor to describe the effect of high-altitude adaptation on the $\dot{V}O_2$peak.

Based on the lung volume results reported previously in these subject groups (Brutsaert et al., 1999), the hypothesis of a functional correlation between lung size and $\dot{V}O_2$peak in hypoxia is also tested. A strong correlation can imply the following: at the individual level, functional correlation implies that pulmonary diffusion capacity is that component of the oxygen transport chain which limits $\dot{V}O_2$peak. This is of intrinsic interest to the physiologist (see Piiper and Scheid, 1981), but is not the focus of this study. At the population level, if genetic effects are demonstrated for both lung size and $\dot{V}O_2$peak phenotypes, then functional correlation between the two implies that natural selection has acted on the work capacity phenotype by selecting individuals with larger lungs, i.e., larger pulmonary diffusion capacities. The latter is often implied in studies of HANs, but we are only aware of one instance in the literature where this hypothesis has been directly tested (Sun et al., 1990).

METHODS

Subjects and study design

Nonsmoking male subjects between ages 18–35 years were recruited by newspaper advertisement, and through contact with health professionals, university administrators, and community leaders. All subjects were in good health and gave consent after being informed of the risks and benefits associated with the study. The study protocol was approved by the Cornell University Human Subjects Committee, and the Human Subjects Committee of the Instituto Boliviano de Biología de Altura (IBBA), in La Paz, Bolivia.

A complete migrant study design was employed with 8 distinct subject groups, as shown in Figure 1. Groups were studied at high altitude (which included two testing sites, La Paz, Bolivia, 3,600 m, and Pucarani, Bolivia, 3,850 m) and low altitude (Santa Cruz, Bolivia, 420 m), and were defined based on their ancestral exposure to high altitude as well as on migration status.

Subjects with an ancestral exposure to high altitude were termed "high-altitude natives" (HANs, Aymara and/or Quechua ancestry), and subjects with no ancestral exposure to high altitude were termed "low-altitude natives" (LANs, European and/or North American ancestry). HANs are thus considered the descendants of Amerindians who have had a history of sustained habitation in the high Andes region of approximately 10,000 years (Cardich, 1994). For both HANs and LANs, three distinct groups of migrants were defined: adult migrants were born and raised in their native environment and migrated as adults (>18 years old) to the nonnative environment. Adult migrants had at least 2 months of acclimatization time to the nonnative environment prior to the study (ranging to a maximum exposure of more than 5 years in a few subjects). Thus, adult migrant subjects were studied at a time after ventilatory and hematological acclimatization to high altitude (Huang et al., 1984), and after a period of deacclimatization when studied at low altitude. Child migrants were subjects born in their native environment who had migrated to the nonnative environment during the period of growth and development (between 0–18 years). Multigeneration migrants were defined as subjects born and raised in a nonnative environment as either first-generation migrants, or as the children or grandchildren of previous migrants. Developmental exposure to high altitude depends on migration status, as shown in Figure 1.

For each subject, four surnames were obtained: paternal, mother's maiden name, paternal grandmothers maiden name, and the maternal grandmother's maiden name. This information has been used previously as an indicator of population admixture in the Andes (Greksa, 1992). Surname analysis was used as a criterion for subject selection in LANs only, i.e., LAN subjects were not admitted into the study if they had even one surname of Aymara or Quechua origin. This was an important selection criterion for LAN child migrants and multigeneration LAN migrants born and raised at high altitude (HA), as these groups have a high potential for admixture with indigenous groups.

HANs born and raised at high altitude (HAN-BHA, N = 21). These subjects were studied in their native environment and were rural Aymara-speaking Amerindians living on the Bolivian *altiplano* in the village of Pucarani, ~10 miles from the Southern shore of Lake Titicaca. These subjects were subsistence farmers, raising food crops and tending flocks of domesticated animals. Large amounts of European admixture are not likely for rural Andean populations that still practice a traditional lifestyle. Sixty-three percent of the surnames in this group were of Aymara or Quechua origin, with the remaining names of Spanish origin.

LAN adult migrants to high altitude (LAN-AMHA, N = 24). These subjects were expatriate European and North American professionals born and raised at low altitude and living and working in La Paz, Bolivia. Subject interviews established that this was a rather physically active group. Recreational activities included mountain climbing, mountain biking, and trekking. Admixture with Amerindian populations was not considered a problem in this subject group, and no surnames were of Aymara or Quechua origin.

LAN child migrants to high altitude (LAN-CMHA, N = 9). These subjects were born at low altitude and migrated to high altitude as children. The mean age of migration was at 9.7 ± 4.7 (S.E.) years. They were recruited from the private university system in La Paz, Bolivia, and as such were generally from the upper socioeconomic class of the city. These subjects came from families of European or North American ancestry who had migrated to La Paz for business and/or diplomatic associations with the Bolivian community. Physical activity levels in this group varied from sedentary subjects to physically active subjects who participated in organized sport. We were only able to locate 9 such subjects willing to participate in the study. No surnames in this group were of Aymara or Quechua origin.

LANs born and raised at HA (LAN-BHA, N = 19). These subjects were multigeneration migrants to high altitude and came from established North American and/or

European migrant communities in La Paz. These subjects were also recruited from the private university system. Family history interviews were used to establish no admixture with Andean populations. Most of the subjects were the children or grandchildren of previous migrants, but some subjects could trace their ancestry at high altitude back two or more generations. Thus, historic high-altitude exposure varied in this group. Physical activity patterns also varied within this group, but were generally similar to patterns observed in the LAN-CMHA group. No surnames in this group were of Aymara or Quechua origin.

LANs born at low altitude (LAN-BLA, N = 23). These subjects were tested in their native environment and were upper-class Brazilians of European ancestry who were attending medical school in Santa Cruz. In general, these subjects described themselves as being sedentary. No surnames in this group were of Aymara or Quechua origin.

HAN adult migrants to low altitude (HAN-AMLA, N = 20). These subjects were Aymara- or Quechua-speaking Amerindians who were born and raised at high altitude. Many of these migrants were urban rather than rural residents of the *altiplano* prior to their migration. Thus, we expect greater European admixture in this group than in the rural Aymara group tested at high altitude. These subjects were generally young men in search of work and they occupied a very low position on the Bolivian socioeconomic scale. Most described themselves as sedentary, and manual laborers were rare in the sample. Many potential subjects in this group were ultimately excluded from the sample because of obvious health problems which included anemia (hemoglobin <13.4 g/dl), respiratory problems related to mining work, and/or an inability to complete the exercise test. Forty-eight percent of the surnames in this group were of Aymara or Quechua origin, with the remaining surnames of Spanish origin.

HAN child migrants to low altitude (HAN-CMLA, N = 18). These subjects were born at high altitude and migrated to low altitude as children. The mean age of migration was at 9.8 ± 5.2 (S.E.) years. In addition to age of exposure to low altitude, they differed from the HAN-AMLA group in that they were not a transient group, but rather came from established neighborhoods in Santa Cruz. Most *altiplano* migrant communities in Santa Cruz can be characterized as impoverished, but health and nutrition status is better than in transient groups. While physical activity varied in this group, the majority of subjects described themselves as sedentary. Thirty-three percent of the surnames in this group were of Aymara or Quechua origin, with the remaining surnames of Spanish origin.

HANs born and raised at low altitude (HAN-BLA, N = 16). These subjects were multigeneration migrants to low altitude and were similar in socioeconomic status to the HAN-CMLA group. Migration from the *altiplano* to Santa Cruz, Bolivia is a relatively recent phenomenon, and most subjects were the children or grandchildren of earlier migrants. For this reason, significant admixture with lowland populations was considered unlikely. However, only 28% of the surnames in this group were of Aymara or Quechua origin, with the remaining surnames of Spanish origin.

Anthropometry, hematology, and pulmonary function

Subjects were measured using standard anthropometric techniques (Weiner and Lourie, 1981) by the same investigator. Height, weight, percent body fat (%body fat), and fat-free mass (FFM) are reported here. The %body fat and FFM were calculated from skinfold measurements (subscapular, suprailiac, biceps, and triceps) according to equations given by Durnin and Womersley (1974). Hemoglobin concentration (Hb) was measured by a Hemocue blood hemoglobin analyzer (Hemocue AB, Angelholm, Sweden) from capillary blood obtained by finger prick. Pulmonary function was assessed with a Collins 9 Liter Survey Spirometer (Warren Collins, Braintree, MA). Each subject performed a maximal inspiration, followed immediately by a forced maximal expiration while in a seated position. From this proce-

dure, the forced vital capacity (FVC) was determined based on the best of at least two efforts. FVC was corrected for body temperature, pressure, saturation (BTPS).

Exercise test locations and conditions

The HAN subjects tested at high altitude were tested in the village of Pucarani, Bolivia, situated at 3,850 m above sea level, with a mean barometric pressure and temperature during the study of 471 mm Hg and 17°C, respectively. The LAN subjects tested at HA were tested at the Instituto Boliviano de Biologia de Altura (IBBA) in La Paz, Bolivia, situated at 3,600 m, with a mean barometric pressure and temperature during the study of 499 mm Hg and 19°C, respectively. Because of the altitude difference between the two test locations, the $\dot{V}O_2$peak values of the HAN group were corrected (increased by 2.67%) according to the altitude correction factor given by Squires and Buskirk (1982) for the expected 3.2% decrease in aerobic capacity per 300 m above 1,500 m. All subjects tested at low altitude were tested at the Centro de Enfermedades Tropicales (CENETROP) in Santa Cruz, Bolivia, situated at 420 m, with a mean barometric pressure and temperature during the study of 725 mm Hg and 27°C, respectively.

$\dot{V}O_2$peak testing

$\dot{V}O_2$peak testing took place according to standard protocols on a mechanically braked cycle ergometer as an incremented series of increasing work loads of 3-min duration each until subject exhaustion. The typical $\dot{V}O_2$peak test lasted from 10–15 min. During testing, heart rate (HR) was continuously monitored with a Vantage XL Polar Heart Rate Monitor (Electric Oy, Oulu, Finland). During exercise testing, subjects inspired room air through a low-resistance breathing valve. Expired fractions of O_2 and CO_2 were measured continuously from a mixing chamber (Applied electrochemistry S-3A oxygen analyzer [Sunnyvale, CA] and a Beckman LB-2 CO_2 analyzer [Beckman Cardiopulmonary Instruments, Fullerton, CA], respectively). Gas analyzers were calibrated to gas standards prior to each test. Inspired minute ventilation (VE-ATP) was measured by a dry gas meter (Rayfield Electronics, Chicago, IL), which was calibrated with a 3-liter calibration syringe. These data were processed by an automated O_2 uptake system (REP-200B, Rayfield Electronics) to produce 30-sec-interval calculations of $\dot{V}O_2$, carbon dioxide production ($\dot{V}CO_2$), and minute ventilation ($\dot{V}$E-BTPS). Respiratory exchange ratio (RER) and ventilatory equivalent ($\dot{V}E/\dot{V}O_2$) were calculated from these data. Although subjects were verbally encouraged to achieve maximum work output, the objective criteria for a true $\dot{V}O_2$peak included a maximal heart rate (HR) within 5% of the age predicted maximum (220 bpm minus age in years) and/or an RER greater than 1.1. There were no significant differences in the HR and RER at maximal exercise between HANs and LANs tested within a given environment to indicate group differences in achieving $\dot{V}O_2$peak.

Submaximal exercise testing and construction of a training level index

To measure the HR response during submaximal exercise and recovery, subjects were given two separate submaximal tests, each of which consisted of four 5-min work loads. Work loads were set at approximately 40%, 50%, 60%, and 70% of observed $\dot{V}O_2$peak, and were separated by 5-min rest periods. Figure 2 shows the HR curve during a submaximal exercise protocol for one study subject. During a 5-min work bout, the HR increases from rest to a steady-state level after 2–3 min. At the completion of work, the HR falls as an exponential during a rapid deceleration phase lasting approximately 2 min (Kirby and Hartung, 1980). After this rapid phase, deceleration is slower and HR approaches a new "resting" level within 5 min. For subsequent work loads, the HR rises again from this resting level to a new steady state, and so forth.

Two parameters of this HR dynamic were used in the construction of the training level index: 1) the *percent recovery of HR* after each work bout (calculated as the percent return of HR after 1 min to a resting level measured 5 min post-exercise), and 2) the *rate of increase of the post-exercise resting HR* (calculated as the slope of the line describing the increase of the post-exercise

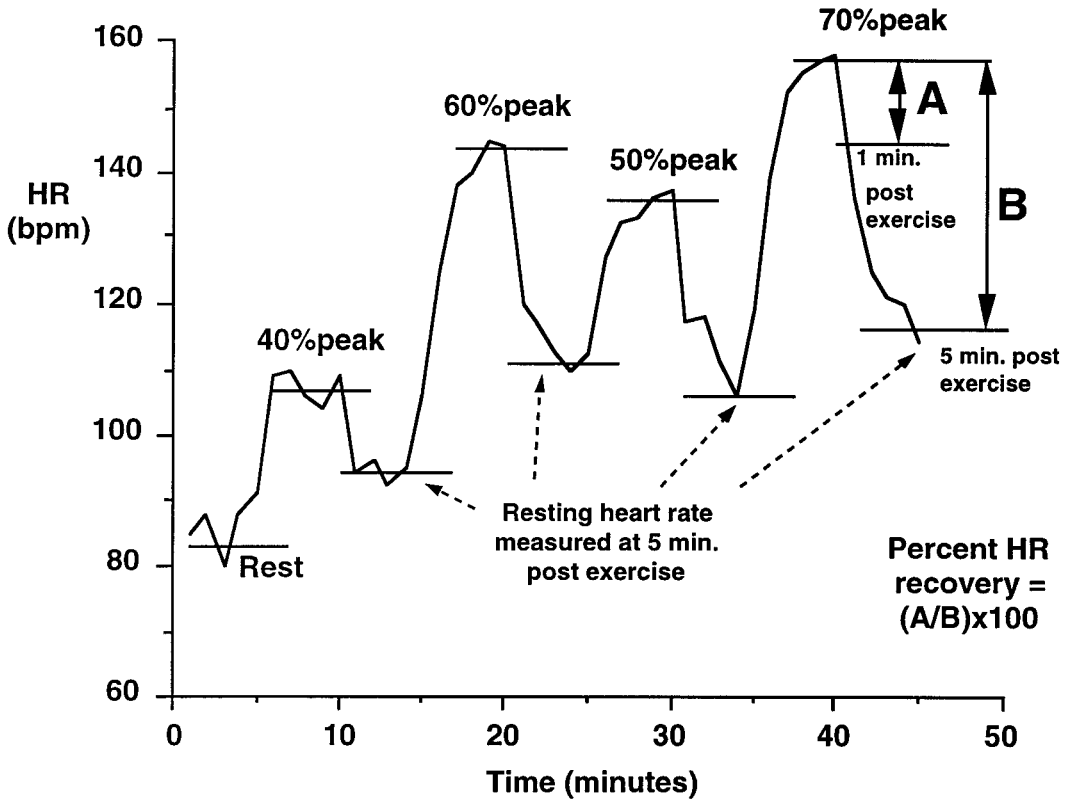

Fig. 2. Heart rate (HR) curve for one study subject during a submaximal exercise test. Percent HR recovery 1 min following exercise after each work bout (~40%, 50%, 60%, and 70% $\dot{V}O_2$peak) was calculated as shown. The rate of increase of post-exercise resting HR was calculated as the slope of the line describing the relationship between post-exercise resting HR vs. the %$\dot{V}O_2$peak preceding the rest period.

resting HR as a function of increasing relative work measured after workloads 1–4).

The training level index was constructed for each individual as a sum of the components parts described from the two separate submaximal tests, where each component part was first standardized relative to the overall group mean. For example, the standardized percent of HR recovery after work bout 1 was calculated as the difference between the individual and overall group mean percent HR recovery at that work load, divided by the standard deviation of percent HR recovery. This value was added to the standardized percent HR recovery after work bout 2, and so forth. The rate of post-exercise HR increase was entered as a negative value because of the negative relationship between this value and the training status of the individual. This process gives a unitless index which can be used for comparing the HR recovery (and thus the training status) of individuals and groups within the study.

Validity of the training level index

No longitudinal data were collected to validate the training level index. However, index validity can be established by consideration of the physiological principle that underlies its construction. It is well-known that a trained individual shows a more rapid and a more complete return of the HR to a resting level after exercise compared to an untrained individual (Cardus and Spencer, 1976; Kirby and Hartung, 1980; Darr et al., 1988). This principle has been used in the past to construct an index of training level shown to be valid (see Kirby and Hartung, 1980).

Another means of assessing the validity of the index is to "test" its utility as a covariate

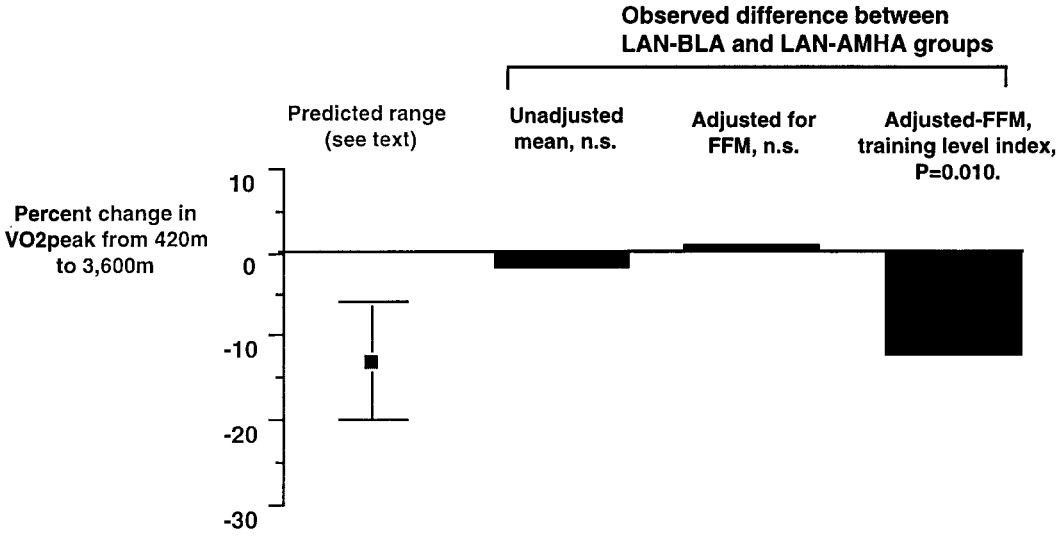

Fig. 3. The $\dot{V}O_2$peak decrement with altitude exposure (% decrease from 420 m to 3,600 m) modeled as the difference in $\dot{V}O_2$peak between the LAN-BLA vs. LAN-AMHA groups who were tested at 420 m and 3,600 m, respectively. These results are compared to the $\dot{V}O_2$peak decrement expected due to acute altitude exposure in hematologically acclimatized subjects (6–20%), and are presented as a means of validating the covariance model expression of $\dot{V}O_2$peak used in this paper to isolate $\dot{V}O_2$peak variability due to high-altitude adaptation (see Methods for details).

in an unrelated statistical model which describes a well-known effect. For example, the effect of acute exposure to altitude on $\dot{V}O_2$peak is well-described, i.e., a mean reduction in $\dot{V}O_2$peak of 22% (range 15–29%) is expected if the same individual is tested at 420 m and at 3,600 m.[1] After hematological acclimatization to high altitude (approximately 2 months), the $\dot{V}O_2$peak improves somewhat. Niu et al. (1995) documented a 9% increase in $\dot{V}O_2$peak in lowland subjects after 7 months at 3,680 m. Thus, it follows that the expected $\dot{V}O_2$peak difference between a lowland subject tested at low altitude and a hematologically acclimatized lowland subject tested at 3,600 m is between 6–20%, all other factors being equal. This is the difference expected between LAN adult migrant subjects tested in La Paz (LAN-AMHA) and LAN subjects tested in Santa Cruz (LAN-BLA), as these two subject groups do not differ in their developmental or ancestral exposure to HA.

A valid index of training level, introduced as a covariate into a statistical model comparing these two LAN groups, should improve the model fit with expectation, particularly if large differences in training level exist between the two groups. This is shown in Figure 3. On the left is the expected $\dot{V}O_2$peak decrement with altitude, given as a range from approximately 6–20%. The $\dot{V}O_2$peak decrement modeled as the difference between the absolute (unadjusted) means of the LAN-AMHA and LAN-BLA groups does not meet expectations, as both groups achieved essentially the same $\dot{V}O_2$peak. Adjusting for FFM differences between groups makes little difference, as groups do not differ greatly in body size or composition. However, adjusting for training level differences between groups brings the model result within the expected range.

In this case, training level differences between the LAN-AMHA and LAN-BLA

[1]There are many studies describing the reduction in $\dot{V}O_2$peak with high altitude. However, an exact model has not been described because the decrement depends on the subject studied. For example, individuals with a high $\dot{V}O_2$peak at sea level tend to show the largest decrement in $\dot{V}O_2$peak at high altitude (Robergs et al., 1998). To establish an expected $\dot{V}O_2$peak decrement from 420 m to 3,600 m, data compiled by Robergs and Roberts (1997) from 20 studies of acute altitude exposure were used. Their model indicates a mean expected $\dot{V}O_2$peak decrement at 3,600 m of 22% (range, 15–29%) in nonacclimatized subjects. The range presented in this paper also takes into account the improvement in $\dot{V}O_2$peak expected after hematological acclimatization.

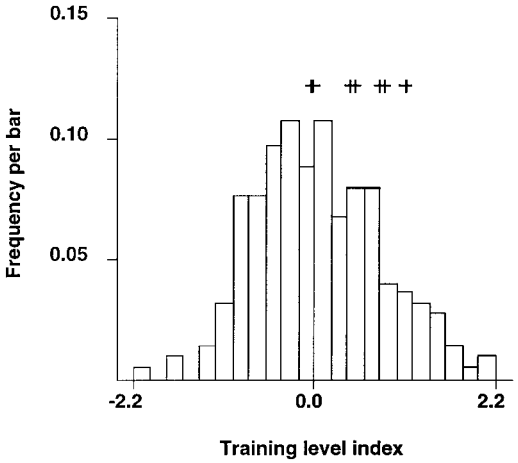

Fig. 4. Frequency distribution of training level index for the entire study sample. +, individual data points for the training level index calculated on the same subject weekly over 8 weeks. Retest variability is 24.5% of the total variability, suggesting that the training level index reliably places a given subject within a specific quartile of the overall frequency distribution.

groups confound the ability to describe the "true" $\dot{V}O_2$peak difference due to hypoxia in hematologically acclimatized subjects. Recall that the LAN-BLA subjects (tested at 420 m) were sedentary medical students, which explains why their unadjusted mean $\dot{V}O_2$peak measured at 420 m was no higher than that measured in the LAN-AMHA subjects at 3,600 m who were highly active. This demonstrates both the validity and utility of the training level index. In the analytic models which follow, the training level index is used as a covariate to remove the effect of training level differences between groups, so that the putative effects of genetic and/or developmental adaptation on the $\dot{V}O_2$peak phenotype may be correctly described.

Reliability of the training level index

One individual subject was tested weekly over a 2-month period to produce eight calculations of the training level index. This subject maintained a constant level of physical activity throughout. Figure 4 shows the calculated values of the training level index (+ signs) obtained for this subject during the test-retest period superimposed on the frequency distribution of the index for the entire study sample. The retest variability was 24.5% of the total variability in the training level index. This suggests that the index reliably places an individual subject within a specific quartile of the overall index distribution.

Analytic models and statistics

Anthropometric and hematological variables were tested for group differences by analysis of variance (ANOVA). FVC differences were tested by analysis of covariance (ANCOVA), adjusting for stature, according to the model described previously (Brutsaert et al., 1999). Statistical significance testing for the genetic and/or developmental effects of high-altitude exposure on $\dot{V}O_2$peak was by ANCOVA adjusting for FFM and the training level index. In all models, the FFM and training level index were significantly related to the $\dot{V}O_2$peak in independent regressions. For example, in those groups used to test for genetic effects, FFM ($P = 0.002$, $R^2 = 0.12$) and the training level index ($P < 0.000$, $R^2 = 0.14$) explained a significant amount of the variability in the $\dot{V}O_2$peak.

The developmental effects model was a single-factor ANCOVA, with developmental high-altitude exposure tested as the main effect across migrant groups of LANs at high altitude (LAN-AMHA, LAN-CMHA, and LAN-BHA). Developmental effects were tested across LAN migrant groups at high altitude only, as the $\dot{V}O_2$peak phenotype is conceptualized as an environment-specific phenotype, i.e., $\dot{V}O_2$peak in hypoxia. This is unlike the case for lung volumes, where the phenotype can be considered as independent of environment, and where it is possible to test for developmental by ancestral (genetic) interactions (see Brutsaert et al., 1999). Three levels of developmental exposure in this model were defined according to migration status as: 1) no developmental exposure; 2) partial developmental exposure; and 3) full developmental exposure. The genetic effects model was a single-factor ANCOVA, with ancestral HA exposure group (HAN vs. LAN) tested as the main effect. This model holds the effect of developmental exposure constant by comparing HAN and LAN subject groups who were born, raised, and tested at high altitude (i.e., the HAN-BHA and LAN-BHA subject groups).

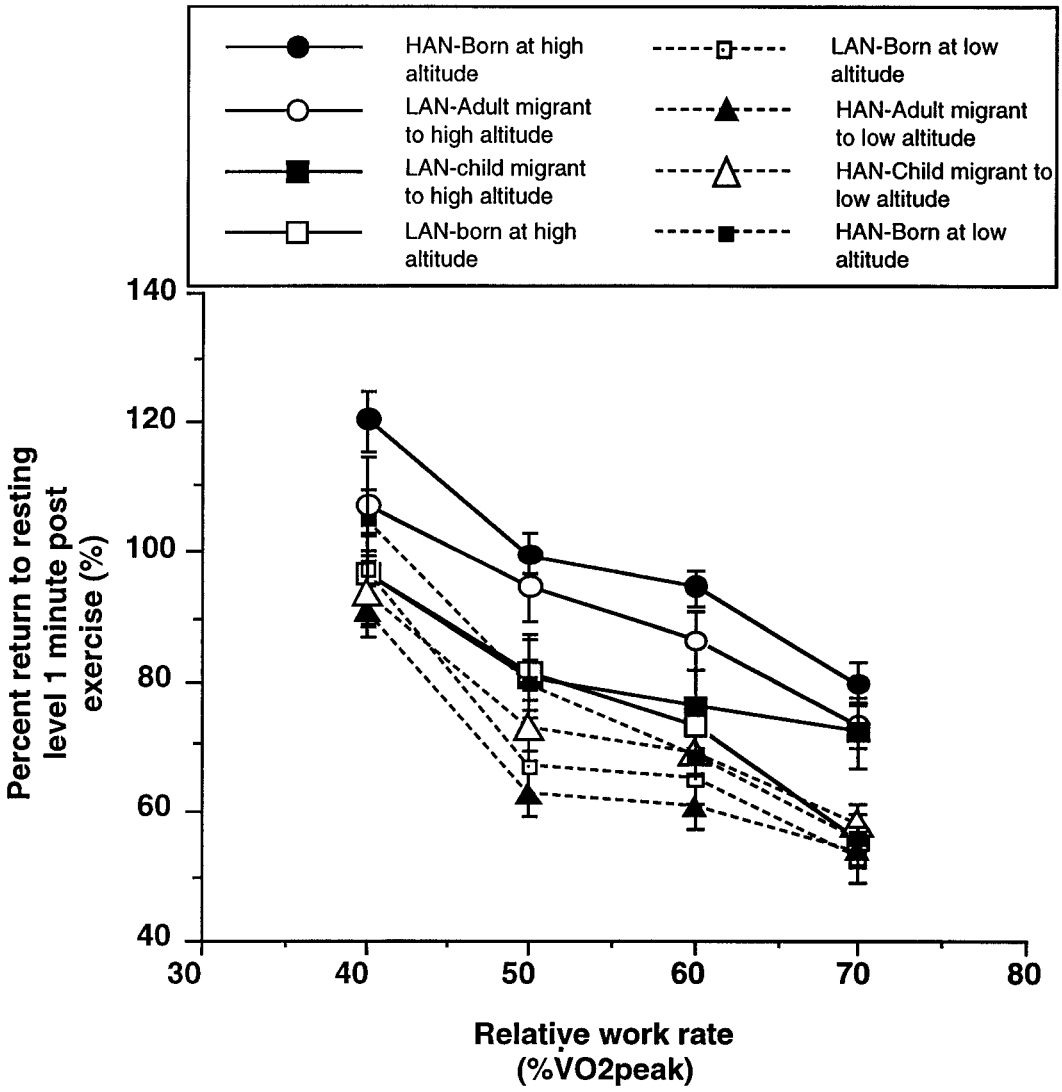

Fig. 5. Mean percent HR recovery by groups 1 min after completion of 5-min workloads given at ~40%, 50%, 60%, and 70% $\dot{V}O_2$peak. Standard errors are shown.

In all models, statistical significance for main effects was assumed at the $P \leq 0.05$ level. All statistics were performed using the general linear model (GLM) procedure of Systat Statistical Software, version 5.2 (Evanston, IL).

RESULTS

Data used in the construction of the training level index

Because of the importance of the training level index in the analyses which follow, data which were used to construct this index are presented first. Figure 5 shows the group mean HR recoveries following submaximal exercise. HANs born and raised at high altitude had the most rapid return of HR to a resting level at all work loads compared to other study groups, indicating a greater level of cardio-respiratory fitness due to high levels of physical activity (training). LAN adult migrants to high altitude also showed a rapid return to a resting level post-exercise, while HRs in most groups studied

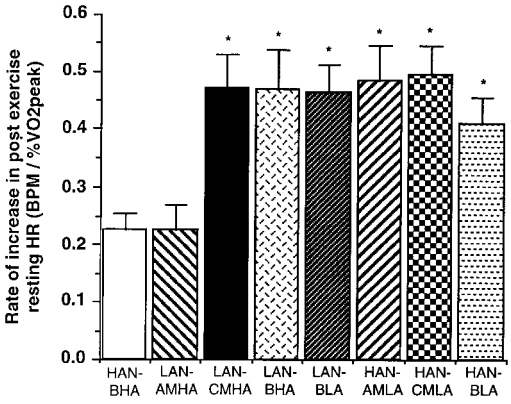

Fig. 6. Mean rate of increase in post-exercise resting heart rate (HR) by study group (bpm/%$\dot{V}O_2$peak). Standard errors are shown. *Significantly different from HAN-BHA group, $P \leq 0.05$.

at low altitude (Fig. 5, dashed lines) were relatively higher at 1 min post-exercise. Figure 6 shows the overall rate of increase (slope) in the post-exercise resting HR from 40% $\dot{V}O_2$peak to 70% $\dot{V}O_2$peak. This rate can be interpreted as the expected increase in post-exercise resting HR per unit increase in the relative work output performed prior to rest. It is clear that HANs born and raised at high altitude and LAN adult migrants to high altitude also had a smaller rate of increase in resting HR post-exercise compared to the other groups in the study. The calculated levels of the training level index for each group are given in Table 1. The highest levels of the training level index were obtained in the HAN-BHA (0.91) and LAN-AMHA (0.62) groups. The lowest level of the training level index was obtained in the HAN-AMLA group (-0.58). These levels of the training level index corresponded well to subjective impressions of group physical activity level from subject interviews.

Group characteristics

A summary of study group characteristics, including anthropometric, hematologic, and pulmonary function characteristics, is given in Table 1. Data presented in this table show mean values, or adjusted mean values from ANCOVA where appropriate. Significance values are given for comparisons of all study groups with two reference groups. Reference groups are defined as the HAN and LAN study groups born and raised in their native environment. Comparisons between migrants and their reference group are presented first, as the general similarity of migrant vs. reference groups is an important consideration of the study design: i.e., migrants are conceptualized as being from the same general population as individuals still residing in their native environment. Comparisons of HANs vs. LANs follow.

HAN and LAN migrant groups compared to their respective reference groups

In general, LAN migrant groups at high altitude were similar to their reference group at low altitude in age, body size, and body composition. However, LAN migrant groups studied at high altitude had a higher Hb level, consistent with a full hematological adaptation to high altitude. LANs born and raised at high altitude, and LAN child migrants to high altitude, had larger FVCs (adjusted for stature) compared to LAN adult migrants at high altitude and LANs tested at low altitude. In our previous paper we concluded that these differences were due to developmental adaptation to high altitude (Brutsaert et al., 1999). LAN adult migrants studied at high altitude had a high group mean training level index, which was consistent with subject interviews revealing high levels of physical activity in this study group. LANs tested at low altitude had a low group mean standardized training level index, consistent with subject interviews in this group revealing that many subjects were sedentary (i.e., medical students).

HAN migrant groups at low altitude were similar to the HAN reference group at high altitude in most respects except for body composition, i.e., HAN migrant groups had a greater percent body fat compared to the HANs at high altitude. HAN migrant groups studied at low altitude had a lower Hb, consistent with a deacclimatization effect. FVC was similar between HANs at high altitude and HAN adult migrants to low altitude. However, FVC was smaller in HAN child migrants to low altitude and HANs born and raised at low altitude compared to the HAN reference group. Again, in our previous paper we showed these differences to be due to the effect of developmental

TABLE 1. Summary group characteristics[1]

	Low-altitude natives (LANs), European/North American			
	Reference group: LANs tested at 420 m (LAN-BLA)	LAN adult migrants to 3,600 m (LAN-AMHA)	LAN child migrants to 3,600 m (LAN-CMHA)	LANs born and raised at 3,600 m (LAN-BHA)
N	23	24	9	19
Age (years)	22.13 (0.94)	27.50 (0.92)*,**	22.22 (1.51)	24.32 (1.04)
Hb (g/dl)	15.31 (0.22)*	17.59 (0.22)*	17.58 (0.33)*	17.81 (0.23)*
Height (cm)	175.28 (1.42)**	176.09 (1.39)**	179.62 (2.28)**	175.36 (1.57)**
Weight (cm)	68.44 (1.77)**	70.22 (1.73)**	73.39 (2.83)**	69.38 (1.95)**
Body fat (%)	20.04 (1.11)**	19.64 (1.09)**	19.59 (1.78)**	20.53 (1.23)**
Fat-free mass (Kg)	54.49 (1.05)**	56.26 (1.03)**	58.56 (1.68)*,**	54.84 (1.15)**
FVC (ml) adjusted height[2]	4,840 (121)**	5,062 (118)**	5,282 (192)*,**	5,334 (133)*,**
Training level index	−0.42 (0.14)**	0.62 (0.14)*	0.07 (0.22)**	−0.15 (0.15)**
	High-altitude natives (HANs)—Aymara/Quechua			
	Reference group: HANs tested at 3,600 m (HAN-BHA)	HAN adult migrants to 420 m (HAN-AMLA)	HAN child migrants to 420 m (HAN-CMLA)	HANs born and raised at 420 m (HAN-BLA)
N	21	20	18	16
Age (years)	23.48 (0.99)	26.45 (1.01)*,***	21.56 (1.07)	20.00 (1.13)
Hb (g/dl)	17.59 (0.22)***	15.19 (0.23)*	14.86 (0.24)*	14.81 (0.25)*
Height (cm)	163.45 (1.49)***	160.17 (1.53)***	62.37 (1.61)***	165.33 (1.71)***
Weight (cm)	56.91 (1.85)***	59.25 (1.90)***	60.08 (2.00)***	63.34 (2.12)*
Body fat (%)	14.90 (1.17)***	20.29 (1.20)*	18.92 (1.26)*	19.67 (1.34)*
Fat-free mass (Kg)	48.29 (1.10)***	47.03 (1.12)***	48.34 (1.19)***	50.53 (1.26)***
FVC (ml) adjusted height[2]	5,649 (117)***	5,397 (119)***	5,139 (121)*,***	4,679 (132)*
Training level index	0.91 (0.15)***	−0.58 (0.15)*	−0.30 (0.15)*	−0.13 (0.16)*

[1] Values are given as means (standard error).
[2] Value given as the adjusted mean from analysis of covariance, controlling for height.
* Significantly different from respective reference group, $P \leq 0.05$.
** Significantly different from HAN-BHA group, $P \leq 0.05$.
*** Significantly different from LAN-BLA group, $P \leq 0.05$.

exposure to high altitude (Brutsaert et al., 1999). HAN migrant groups at low altitude had a much lower group mean standardized training level index compared to the HAN reference group at HA. These group differences in the training level index corresponded well with subject interview and reflected the general lifestyle differences between the HANs studied at high altitude and the migrant groups of HANs studied at low altitude: i.e., HANs studied at high altitude were rural subsistence farmers, while HANs studied at low altitude were sedentary urban dwellers.

HANs vs. LANs

Hemoglobin levels within a given environment were similar between HANs and LANs. However, HANs were smaller (body weight and stature) compared to LANs. The HANs studied at high altitude were significantly leaner than all study groups, but body fat percentage was similar between all LAN groups and the HAN migrant groups at low altitude. HANs at high altitude had significantly larger FVCs (adjusted for stature) compared to LANs born and raised at high altitude. In our previous paper we suggested that this difference is due to genetic adaptation to high altitude in Andean HAN populations (Brutsaert et al., 1999). There are no consistent HAN vs. LAN differences in the training level index, as the variability in this index depends upon lifestyle differences, not differences in ancestry. Thus, the HANs at high altitude and the LAN adult migrant group at high altitude had the highest levels of the training level index compared to all other study groups, consistent with their active lifestyles. In general, the more sedentary population groups, including both HANs and LANs, were those studied at low altitude.

Maximal exercise response

Summary maximal exercise data are given in Table 2. As in Table 1, significance values

TABLE 2. Summary of maximal exercise data[1]

	Low-altitude natives (LANs), European/North American			
	Reference group: LANs tested at 420 m (LAN-BLA)	LAN adult migrants to 3,600 m (LAN-AMHA)	LAN child migrants to 3,600 m (LAN-CMHA)	LANs born and raised at 3,600 m (LAN-BHA)
N	23	24	9	19
$\dot{V}O_2$ ($l \cdot min^{-1}$)	2.91 (0.09)	2.96 (0.09)	2.95 (0.15)	2.82 (0.10)
$\dot{V}O_2$ ($ml \cdot min^{-1} \cdot kg^{-1}$)	42.75 (1.58)**	42.54 (1.55)**	41.30 (2.53)**	41.13 (1.74)**
$\dot{V}O_2$ ($ml \cdot min^{-1} \cdot kg^{-1}$ FFM)	51.58 (1.76)**	52.84 (1.61)**	51.05 (2.63)**	51.43 (1.81)**
$\dot{V}O_2$ ($l \cdot min^{-1}$) (adjusted for FFM and training level)	2.86 (0.08)	2.57 (0.09)*	2.63 (0.13)	2.69 (0.09)
$\dot{V}E$-BTPS	110.69 (4.88)**	152.45 (4.77)*,**	137.47 (7.80)*	132.47 (5.37)*
RER	1.23 (0.03)	1.29 (0.03)	1.28 (0.04)	1.27 (0.03)
HR	195.91 (2.22)**	181.38 (2.17)*	175.78 (3.55)*	185.78 (2.51)*
$\dot{V}E-BTPS/\dot{V}O_2$	38.29 (1.49)**	51.74 (1.46)*,**	46.50 (2.39)*	47.77 (1.64)*
	High-altitude natives (HANs), Aymara/Quechua			
	Reference group: HANs tested at 3,600 m (HAN-BHA)	HAN adult migrants to 420 m (HAN-AMLA)	HAN child migrants to 420 m (HAN-CMLA)	HANs born and raised at 420 m (HAN-BLA)
N	21	20	18	16
$\dot{V}O_2$ ($l \cdot min^{-1}$)	2.94 (0.10)	2.43 (0.10)*,***	2.65 (0.11)*	2.55 (0.11)*,***
$\dot{V}O_2$ ($ml \cdot min^{-1} \cdot kg^{-1}$)	52.03 (1.65)***	41.21 (1.70)*	44.60 (1.79)*	40.83 (1.90)*
$\dot{V}O_2$ ($ml \cdot min^{-1} \cdot kg^{-1}$ FFM)	60.97 (1.72)***	51.58 (1.76)*	54.92 (1.86)*	50.72 (1.97)*
$\dot{V}O_2$ ($l \cdot min^{-1}$) (adjusted for FFM and training level)	2.73 (0.09)	2.69 (0.09)	2.79 (0.09)	2.57 (0.09)*
$\dot{V}E$-BTPS	131.68 (5.10)***	97.54 (5.23)*	108.74 (5.51)*	96.41 (5.85)*
RER	1.30 (0.03)	1.21 (0.03)*	1.31 (0.03)**	1.31 (0.04)
HR	182.38 (2.32)***	188.60 (2.38)***	187.41 (2.58)***	192.2 (2.75)*
$\dot{V}E-BTPS/\dot{V}O_2$	44.89 (1.56)***	40.30 (1.60)*	41.37 (1.69)	37.87 (1.79)*

[1] Values are given as mean (standard error) or as least square adjusted mean from analysis of covariance where indicated.
* Significantly different from respective reference group, $P < 0.05$.
** Significantly different from HAN-BHA group, $P < 0.05$.
*** Significantly different from LAN-BLA group, $P < 0.05$.

are given for comparisons with two reference groups. These data are meant to give an overview of the maximal exercise response for all study groups. The more relevant comparisons related to the goals of this study are presented below as the results of covariance modeling to test for developmental and/or genetic effects, using specific study group subsets as described in the methods.

In Table 2, $\dot{V}O_2$peak is expressed in absolute terms ($l \cdot min^{-1}$), as a ratio standard to body weight ($ml \cdot min^{-1} \cdot kg^{-1}$), a ratio standard to fat free mass ($ml \cdot min^{-1} \cdot kg^{-1}$ FFM), and as the adjusted mean ($l \cdot min^{-1}$) by covariance analysis, taking into account the FFM and training level differences between study groups. In general, it should be noted that the magnitude of group difference depends upon how the $\dot{V}O_2$peak is expressed. For example, the $\dot{V}O_2$peak difference between HANs at high altitude and LAN adult migrants to high altitude is large when expressed as $ml \cdot min^{-1} \cdot kg^{-1}$ and decreases significantly when expressed as a difference between adjusted means ($l \cdot min^{-1}$ adjusted for FFM and training level index). As expected, the respiratory exchange ratio at maximal exercise was similar between all study groups, and ventilation and ventilatory equivalent were higher in groups tested at high altitude. Ventilatory response differences between groups related to developmental and/or ancestral (genetic) high-altitude exposure are discussed below.

Developmental adaptation

Figure 7A shows the results of covariance modeling for the developmental effect of HA exposure on $\dot{V}O_2$peak (adjusting for FFM and training level index) across LAN migrant groups tested at high altitude. The mean values of $\dot{V}O_2$peak for a given group

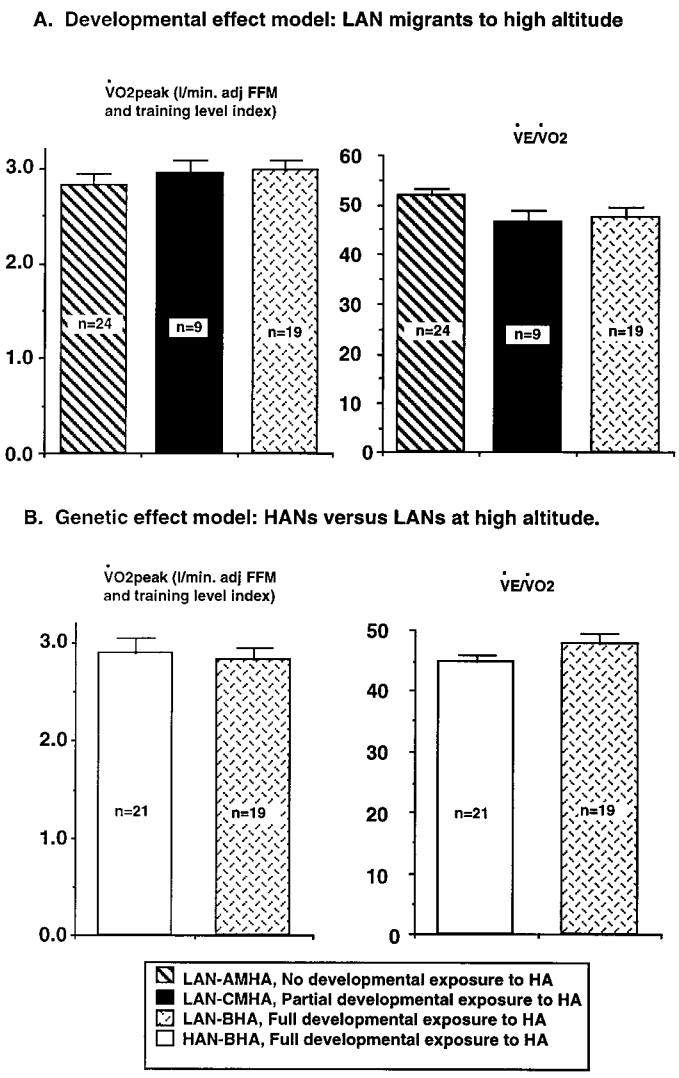

Fig. 7. Covariance model expressions of (**A**) the developmental effect of high-altitude exposure, and (**B**) the ancestral (genetic) effect of high-altitude exposure for the $\dot{V}O_2$peak and the $\dot{V}E/\dot{V}O_2$. Developmental effects were tested across LAN migrant groups at high altitude who differed with developmental exposure to high altitude, ranging from none, to partial, to full exposure. Genetic effects were tested as differences between HANs and LANs born, raised, and tested at high altitude. No significant developmental or genetic effects were detected at $P \leq 0.05$. adj, adjusted.

differ slightly from those given in Table 2 for the same subject groups, as they reflect the least square means from a different ANCOVA model. Although there is a trend for increasing $\dot{V}O_2$peak with length of developmental exposure to hypoxia, the effect did not reach statistical significance ($P = 0.613$). As a ratio, $\dot{V}E/\dot{V}O_2$ at maximal exercise should not be confounded by body size or training level considerations. A stronger developmental trend was detected for $\dot{V}E/\dot{V}O_2$, but again this trend did not reach statistical significance ($P = 0.077$). No developmental effects were detected for HR at maximal exercise or RER.

Genetic adaptation

Figure 7B shows results from covariance modeling for the genetic effect of high-altitude exposure, comparing HANs and LANs, born, raised, and tested at high altitude. Again, group mean values differ slightly from those given in Table 2, reflecting the different ANCOVA model. No difference was detected between these two study groups for the $\dot{V}O_2$peak (adjusted for FFM and training

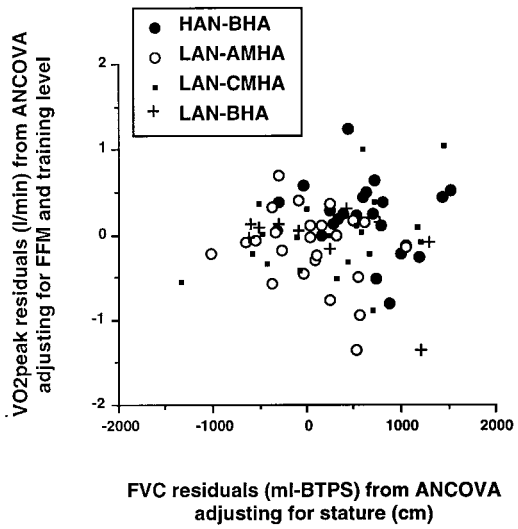

Fig. 8. There is no functional relationship between $\dot{V}O_2$peak ($l \cdot min^{-1}$, adjusting for FFM and training level) and FVC (ml-BTPS, adjusting for stature), either across groups or within groups.

level) or for the $\dot{V}E/\dot{V}O_2$. Further, there were no group differences apparent for HR or RER at maximal exercise.

Functional correlation between $\dot{V}O_2$peak and FVC

Figure 8 shows the relationship between the FVC residuals from ANCOVA (adjusting for height) and the $\dot{V}O_2$peak residuals from ANCOVA (adjusting for FFM and training level index) for the four groups studied at high altitude. Plotting the residuals avoids the bias inherent in ratio standard expressions of $\dot{V}O_2$peak and FVC. Although a weak relationship is evident across groups, especially for the LAN adult migrants and HANs born and raised at high altitude, this relationship was not statistically significant by regression analysis. There was also no significant correlation evident within any of the study groups presented. Similar results (not shown) were obtained irrespective of the manner in which the $\dot{V}O_2$peak was expressed.

DISCUSSION

From a comparative study of HANs and LANs in the Bolivian Andes, results are presented from covariance analyses which express the variability in $\dot{V}O_2$peak due to high-altitude adaptation (at the genetic and/or developmental levels), by controlling for known differences between HANs and LANs in body size, body composition, and physical activity level (training). While data are presented for HANs and LANs at both high and low altitude, analysis focuses primarily on groups tested at high altitude. This is because it is assumed that the $\dot{V}O_2$peak phenotype is environment-specific, i.e., if adaptive change to high-altitude exposure has occurred in one or more components of the oxygen transport system, it is assumed that such change has little effect on $\dot{V}O_2$peak in normoxia. This assumption is supported by previous studies (Way, 1976; Hochachka et al., 1991), and by results presented here, which show that HANs born at high altitude and tested at low altitude have a $\dot{V}O_2$peak which is similar to (or no higher than) that of low-altitude natives in normoxia.

No effect of developmental adaptation on $\dot{V}O_2$peak in hypoxia was detected, nor was there any difference in $\dot{V}O_2$peak between HAN and LAN groups born, raised, and tested at high altitude to suggest a genetic effect of high-altitude exposure. Sample size limitations may have precluded an ability to detect significant adaptation effects, particularly in the case of developmental adaptation, but the lack of significant findings should be considered in light of the following: 1) previously in these same subject groups (and thus with the same sample sizes), high-altitude adaptation (both developmental and genetic) was shown through an increase in lung volume (Brutsaert et al., 1999); and 2) in the present study, despite large lungs in HANs, there is no functional correlation between FVC and $\dot{V}O_2$peak within or between groups. These issues are discussed further, following a discussion of the validity of the study design and analytic approach.

Validity of study design

Interpretation of these findings depends greatly on the validity of the study design, which itself depends on proper designation

of individuals into study groups. The potential problem in this regard is admixture between European and Andean populations (Chakraborty et al., 1989). In the present study, the largest concern was for admixture of LAN migrant groups with indigenous groups. We did not admit any LAN migrants into the study if they had an Aymara or Quechua surname. Similarly, the LAN Brazilians studied at low altitude were only studied if a surname analysis revealed no Aymara or Quechua surnames. Thus, for LAN groups, admixture is not considered a potential confounder.

The other principle concern was admixture of the HANs studied at high altitude with European populations. Rural Aymara were deliberately chosen in this regard, as admixture is less of a problem than in urban populations. Despite the likelihood of at least some admixture, the analytic model remains valid: i.e., even with admixture, Aymara or Quechua populations can still be viewed as "much more" Aymara or Quechua than appropriately chosen comparison groups with no indigenous admixture.

A final, but less crucial problem concerns admixture in HANs migrant to the lowlands. These migrant groups are not an important part of the analyses presented in this paper, but it should be noted that they likely differ in admixture rates from the HANs studied at high altitude. As described, HAN migrants were urban dwellers, where higher admixture rates are expected. Despite similarities in body size and in their ability to speak an indigenous language, HAN migrants to low altitude had a lower percentage of Aymara or Quechua surnames compared to rural HANs tested on the Bolivian *altiplano*. Anecdotally, there may be a strong acculturation pressure in the Bolivian lowlands to change one's name from Aymara/Quechua to Spanish.

Validity of statistical models

The covariance approach, used to express $\dot{V}O_2$peak, and controlling for FFM and training level, deserves critical attention. Clearly, $\dot{V}O_2$peak must be normalized to body size, and the potential bias due to body composition difference must be taken into account (Buskirk and Taylor, 1957). Note that LANs in this study were ~12 kg heavier, ~15 cm taller, and had a greater %body fat than HANs. In previous Andean studies, large $\dot{V}O_2$peak differences were reported between Andean HANs vs. LANs when $\dot{V}O_2$peak was expressed as a ratio standard to body weight ($ml \cdot min^{-1} \cdot kg^{-1}$) (Kollias et al., 1968; Mazess, 1969), and when $\dot{V}O_2$peak was expressed as a ratio standard to FFM ($ml \cdot min^{-1} \cdot kg^{-1}$ FFM) (Frisancho et al., 1995). Unfortunately, the ratio standard approach itself is biased in that it favors individuals of a small body size (Nevill et al., 1992; Tanner, 1949). Statistical control for FFM accomplishes two goals simultaneously: it provides a nonbiased expression of group difference, and it eliminates bias which may be introduced by differences in body composition.

The need to control for physical activity level is clear as well, as training can account for differences in $\dot{V}O_2$peak of 20% or more (Astrand, 1960). In some previous studies of HANs, groups were subjectively matched on the basis of being sedentary (Sun et al., 1990; Ge et al., 1993), or they were categorized as active or inactive based on subject interview (Frisancho et al., 1995). The concepts of "active" or "sedentary" are clearly too broad to be useful when expected $\dot{V}O_2$peak differences are small. In addition, subject recall may be biased, particularly when cultural perceptions of what constitutes work and physical activity differ between groups.

An objective index of physical activity level is desirable. In the present study a *standardized training level index* was constructed from HR recovery data after submaximal exercise, as HR recovery is known to be more rapid and more complete in a well-trained individual (Cardus and Spencer, 1976; Kirby and Hartung, 1980; Darr et al., 1988). However, single measurements of HR recovery are highly variable and are thus unreliable as measures of training level. In the present study, HR recoveries from eight separate submaximal workloads were obtained from work tests given on separate days. Multiple measurements used in the construct of an index accommodate the potential for error variability in the HR recovery measurement. Figure 4 shows that the training level index is reliable at least to the

extent that it is able to identify the quartile position occupied by an individual study subject in the overall training level distribution. This level of reliability is useful, especially when it is considered that subjective categorizations of physical activity level normally achieve no better resolution than the assignment of subjects into a few categories.

Establishing that the training level index has validity is central to the results described in this paper, i.e., to what extent does the index actually measure the training level effect that it purports to measure? As described in Methods, the longitudinal data necessary to establish validity in this regard do not exist. However, validity can be assessed by consideration of the following: 1) the training level index is based on a sound physiological principle, and similar indices have been shown as valid in the past (Kirby and Hartung, 1980); 2) the index corresponds well to subjective impressions of physical activity level based on subject interviews; and 3) the training level index performs well as a covariate in a statistical model that is unrelated to the major hypotheses of this study. The latter is described in Methods. Essentially, Figure 3 makes clear that the training level index has at least some validity in that it *correctly* adjusts for the training level differences between the LAN-AMHA and LAN-BLA groups, who differed greatly in their physical activity patterns.

When there is uncertainty surrounding the validity of a covariate, the issues of over- or undercontrol in a particular statistical model should be addressed: i.e., does the introduction of a training level covariate eliminate a real difference due to high-altitude adaptation (overcontrol), or does the covariate fail to eliminate the effect of a real confounding factor such as training level (undercontrol)? These issues are examined for the two statistical model results discussed below.

Developmental adaptation

A number of previous studies suggested a developmental effect of high-altitude exposure on $\dot{V}O_2$peak (Mazess, 1969; Grover et al., 1967), with the most direct evidence coming from the work of Frisancho et al. (1973b, 1995), who showed increasing $\dot{V}O_2$peak with length of developmental HA exposure in LANs who had migrated to high altitude as children. We did not have a sufficient sample of LAN child migrants to directly test this hypothesis within the child migrant group (n = 9), and a test of developmental adaptation across migrant groups (Fig. 7A) did not reach statistical significance, despite a clear trend. Because the developmental covariance model increased (rather than decreased) differences between groups compared to the ratio standard approach (ml/min-kg—FFM), overcontrol for covariates was not considered an issue. It is possible that sample size was insufficient to detect a developmental effect across groups, where confounding variability is higher than within groups (which is how this hypothesis has been tested previously). In fact, a power analysis according to Neter et al. (1990) gave only a 50–60% probability of detecting a significant developmental effect of 15% between individuals with a full developmental exposure and no developmental exposure. Despite the likely inability to detect an effect, it can certainly be concluded that developmental effects on maximal oxygen consumption (if they exist) are less than those shown for lung size: i.e., across the same study groups, and thus with the same sample size, we have previously shown a great sensitivity of the lung volume phenotype to developmental high-altitude exposure (Brutsaert et al., 1998). This is clearly not the case in the present study for the $\dot{V}O_2$peak phenotype.

Interestingly, there was a marginally significant effect ($P = 0.077$) of developmental exposure on $\dot{V}E/\dot{V}O_2$ at maximal exercise, i.e., developmentally acclimatized subjects show a lower ventilation for a given level of $\dot{V}O_2$ at maximal work output. Schoene et al. (1990) showed that despite a lower ventilation during exercise at high altitude, Andean natives are able to maintain the same or higher level of arterial O_2 saturation compared to lowland natives. Thus, it is likely that the lower $\dot{V}E/\dot{V}O_2$ with developmental exposure simply reflects a larger pulmonary diffusion capacity, but again this difference is without functional consequence on the $\dot{V}O_2$peak in these subjects.

Genetic adaptation

Only a few previous studies have directly assessed the effect of ancestral HA exposure on $\dot{V}O_2$peak. Frisancho et al. (1995) reported a 15–16% higher $\dot{V}O_2$peak in rural Bolivian Aymara vs. developmentally acclimatized LANs, born, raised, and tested at high altitude. However, $\dot{V}O_2$peak was expressed as ml · min^{-1} · kg^{-1} FFM in that study, and physical activity level was not well-controlled. The studies of Greksa and Haas (1982) and Greksa et al. (1985) were conducted with Aymara children and children of European ancestry born, raised, and tested at high altitude. These studies showed no $\dot{V}O_2$peak differences between HANs and LANs by covariance analysis, although again physical activity was not well-controlled. In the present study, higher $\dot{V}O_2$peak in HANs compared to LANs born, raised, and tested at HA existed only when $\dot{V}O_2$peak was expressed as a ratio standard (ml · min^{-1} · kg^{-1} body weight or ml · min^{-1} · kg^{-1} FFM) (see Table 2), but differences disappeared when FFM and training level differences were statistically controlled (Fig. 7B).

A statistical power calculation for the difference between two independent means (Motulsky, 1995) gives at least a 70% probability to detect a difference as large as that reported previously in the literature. While there may have been insufficient power to detect a smaller difference in $\dot{V}O_2$peak due to genetic adaptation, the model result does suggest that previous reports of genetic adaptation in Andean HANs have been overstated. The possibility of no genetic difference in $\dot{V}O_2$peak between Andean HANs and European LANs must be considered. If true, this would challenge the long-standing hypothesis that Andean HANs are genetically adapted to express a greater physical work capacity in hypoxia (Monge, 1948).

Overcontrol in the choice of covariates (FFM and training level) may be considered an issue, as the covariance model describing group differences in $\dot{V}O_2$peak reduces group differences expressed by the ratio standard approach to a nonsignificant level. However, there are good reasons to think that overcontrol is not the case: 1) it is difficult to argue against the use of the covariance approach to control for body size and body composition differences, given the known bias in the ratio standard approach; 2) as shown, the training level index as a covariate has validity and it actually increases group mean differences in $\dot{V}O_2$peak when entered as a covariate in the developmental effects model; and 3) the highest $\dot{V}O_2$peak value observed in the HAN-BHA group (~65 ml · min^{-1} · kg^{-1}) was not much greater than the highest $\dot{V}O_2$peak value observed in the LAN-BHA group (~63 ml · min^{-1} · kg^{-1}).

Lung size-$\dot{V}O_2$peak functional correlation hypothesis

The lung size-$\dot{V}O_2$peak functional correlation hypothesis essentially holds that the increase in lung size seen in HANs makes possible an increase in $\dot{V}O_2$peak. Support for this hypothesis is mostly conjectural and is based on the following: the relative size of the lung can be used as a proxy measure for the pulmonary diffusion capacity, as these two measures are correlated in both HANs and LANs (Cerney et al., 1973; DeGraff et al., 1970; Dempsey et al., 1971; Guleria et al., 1971; Johnson et al., 1985). While the pulmonary diffusion capacity and $\dot{V}O_2$peak are probably not causally related in normoxia (Asmussen, 1965), there have been arguments in favor of functional correlation in hypoxia (Piiper and Scheid, 1981). If pulmonary diffusion capacity is a limiting factor which sets the upper limit to $\dot{V}O_2$peak in hypoxia, then it must be the pulmonary diffusion capacity which "adapts" in hypoxia to set new and higher limits for $\dot{V}O_2$peak at the population level. If Andean natives are genetically adapted because of natural selection to express a higher $\dot{V}O_2$peak, then natural selection must have operated on the $\dot{V}O_2$peak phenotype by selecting individuals with larger lungs.

As far as can be determined, this hypothesis has only been explicitly tested once previously (Sun et al., 1990), and these authors showed a positive correlation between vital capacity and $\dot{V}O_2$peak across HAN and LAN groups studied in the Himalayas. In the present study, a test of correlation across the HAN-BHA and LAN-AMHA groups does not reach statistical significance (Fig. 8). Moreover, there is no correla-

tion between FVC and $\dot{V}O_2$peak within groups, which is the more valid approach as it avoids the potential for spurious correlation.

The inability in this study to detect functional correlation between FVC and $\dot{V}O_2$peak is of course consistent with the inability to detect strong developmental and/or genetic effects on the $\dot{V}O_2$peak, despite the strong effects that were detected in the same subjects for the FVC phenotype. If an increase in lung size is the sine qua non of human high-altitude adaptation, then it may be necessary to reevaluate hypotheses concerning adaptive benefit. In other words, if the increase in lung size does not result in an increase in the physical work capacity of the individual, then what adaptive purpose is served? Moore (1990) suggested that maternal oxygen transport to the fetus and fetal growth are key, as these outcomes are more proximal to mortality and morbidity than is $\dot{V}O_2$peak. Others studies suggest that large lungs provide protection against the development of high-altitude pulmonary edema with acute hypoxic exposure (Steinacker et al., 1998; Viswanathan et al., 1969; Podolsky et al., 1996).

Alternately, it may be that there really is a functional correlation between lung size and physical work capacity in hypoxia at the population level. Possibly, the FVC, as a mere proxy for pulmonary diffusion capacity, is not sufficiently sensitive for the relationship to become evident. Resolution of this issue will necessitate the measurement of both pulmonary diffusion capacity and exercise performance ($\dot{V}O_2$peak) in the same samples of HANs and LANs.

CONCLUSIONS

In a previous paper it was concluded that high-altitude adaptation (developmental and genetic) had occurred in this study sample through an increase in lung volume. In the present paper, $\dot{V}O_2$peak variability due to high-altitude adaptation was modeled in the same subject groups by covariance analysis. Despite a clear trend, there was no statistically significant effect of developmental high-altitude exposure on the $\dot{V}O_2$peak. In the developmental model, low statistical power may have limited the ability to detect an effect. In the genetic effect covariance model, HAN and LAN groups born, raised, and tested at high altitude had the same $\dot{V}O_2$peak. In this model, statistical power was likely sufficient to detect an effect at least as large as that reported previously in the literature. Thus, if the $\dot{V}O_2$peak is conceptualized as a proximal measure of individual fitness related to fertility and/or mortality (which is by no means established in the literature), then the results presented in this paper do not support the long-standing hypothesis that Andean HANs have been selected to express a greater physical working capacity in hypoxia. Consistent with this conclusion is the inability to detect a functional correlation between FVC and $\dot{V}O_2$peak, either within or across subject groups tested at 3,600 m.

ACKNOWLEDGMENTS

The authors thank Dr. Enrique Vargas and Dr. Mercedes Villena of the Instituto Boliviano de Biologia de Altura (La Paz, Bolivia), Dr. Alberto Gianella of the Centro de Enfermedades Tropicales (Santa Cruz, Bolivia), and Drs. Tito Cuchallo and Lawrence P. Greksa.

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